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SHORT COMMUNICATION



## Brassinosteroids and sucrose transport in mycorrhizal tomato plants

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### ABSTRACT

Silencing of *SISUT2* expression in tomato plants leads to a dwarfed phenotype, reduced pollen vitality and reduces pollen germination rate. Male sterility of flowers, together with a dwarfed growth behavior is reminiscent to brassinosteroid defective mutant plants. Therefore we aimed to rescue the *SISUT2* silencing phenotype by local brassinosteroid application. The phenotypical effects of *SISUT2* down-regulation could partially be rescued by epi-brassinolide treatment suggesting that *SISUT2* interconnects sucrose partitioning with brassinosteroid signaling. We previously showed that *SISUT2* silenced plants show increased mycorrhization and, this effect was explained by a putative sucrose retrieval function of *SISUT2* at the periarbuscular membrane. More recently, we reported that the symbiotic interaction between Solanaceous hosts and AM fungi is directly affected by watering the roots with epi-brassinolide. Here we show that the *SISUT2* effects on mycorrhiza are not only based on the putative sucrose retrieval function of *SISUT2* at the periarbuscular membrane. Our analyses argue that brassinosteroids as well as *SISUT2* *per se* can impact the arbuscular morphology/architecture and thereby affect the efficiency of nutrient exchange between both symbionts and the mycorrhizal growth benefit for the plant.

### ARTICLE HISTORY

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Mycorrhization; sucrose transporter *SISUT2*; Brassinosteroids; arbuscular anatomy; male sterility

## Introduction

A link between brassinosteroid and primary metabolism was postulated for some time, but the molecular mechanism is still ambiguous. One possible link between BR signaling and carbon metabolism is via the two transcription factors BAM7 and BAM8 containing a BRASSINAZOLE RESISTANT1 (BZR1)-type DNA binding domain on the one side and beta-amylase-like domain on the other. These two transcription factors, BAM7 and BAM8, are able to bind to a BR-responsive cis-regulatory promoter element thereby regulating gene expression. The beta-amylase domain is 1000 times less active in starch breakdown than chloroplastic beta-amylases but it still contains an intact glucan binding site. These transcription factors assumedly affect growth and development by transmitting metabolic signals to BR signaling.<sup>1,2</sup>

We suggest an additional interaction between BR signaling and carbon partitioning at the level of a sucrose transporter. This is based on the fact that the sucrose transporter *SISUT2* of tomato is in direct contact with the BR co-receptor BAK1, the BR signaling inhibitor MSBP1 and with the sterol reductase DIM1 involved in BR biosynthesis.<sup>3,4</sup> We showed recently, that brassinosteroids positively affect the symbiotic interaction between AM fungi and Solanaceous host plants. Overexpression of the MSBP1 inhibitor causes similar morphological modifications on arbuscular anatomy as observed in brassinazole treated plants, an inhibitor of brassinosteroid biosynthesis.<sup>5</sup> Here we provide an additional link between

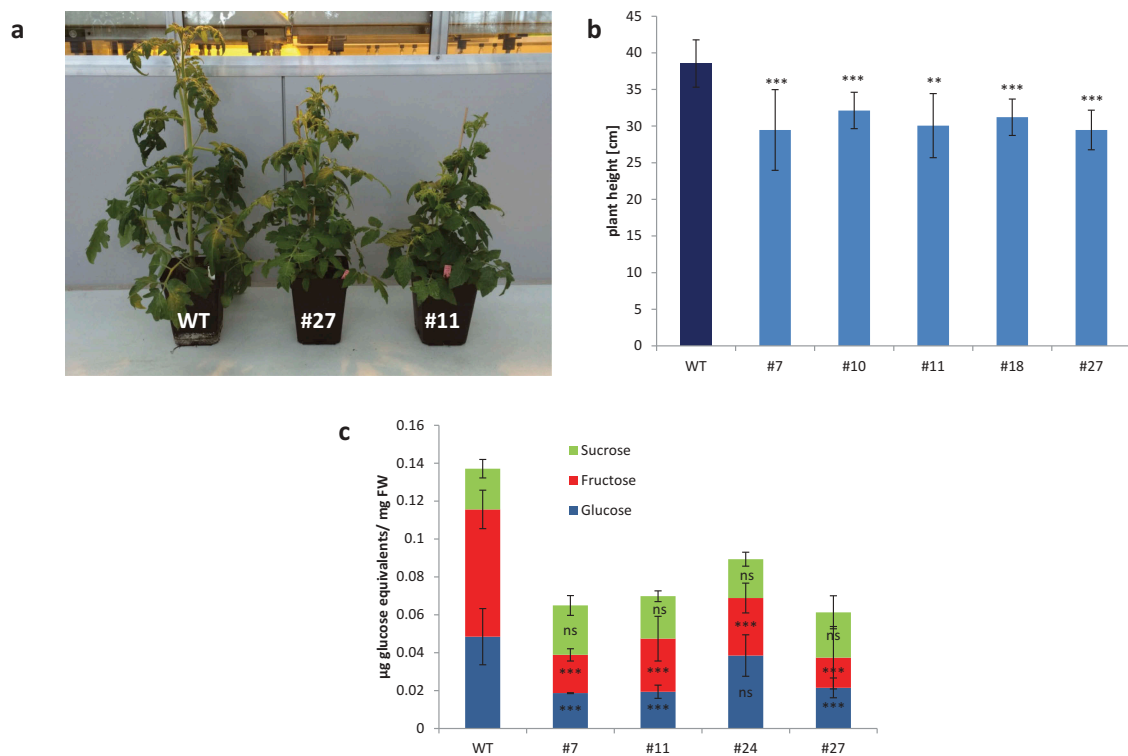
brassinosteroids and sucrose transport by analyzing arbuscular anatomy as well as sugar accumulation in tomato plants with strongly reduced *SISUT2* expression. Interestingly, the phenotypic modification of arbuscular architecture previously described for plants with reduced BR response is also seen in *SISUT2* silenced plants, together with reduced pollen viability and germination rate and a dwarf-like growth behavior.

## Results

Down-regulation of the *SISUT2* expression has led to reduced pollen tube growth, impeded pollen development and reduced tomato fruit yield.<sup>6</sup> Mycorrhization efficiency's increases, when *SISUT2* expression is decreased<sup>3</sup> and we aimed at elucidation of the molecular mechanisms underlying the phenomenon.

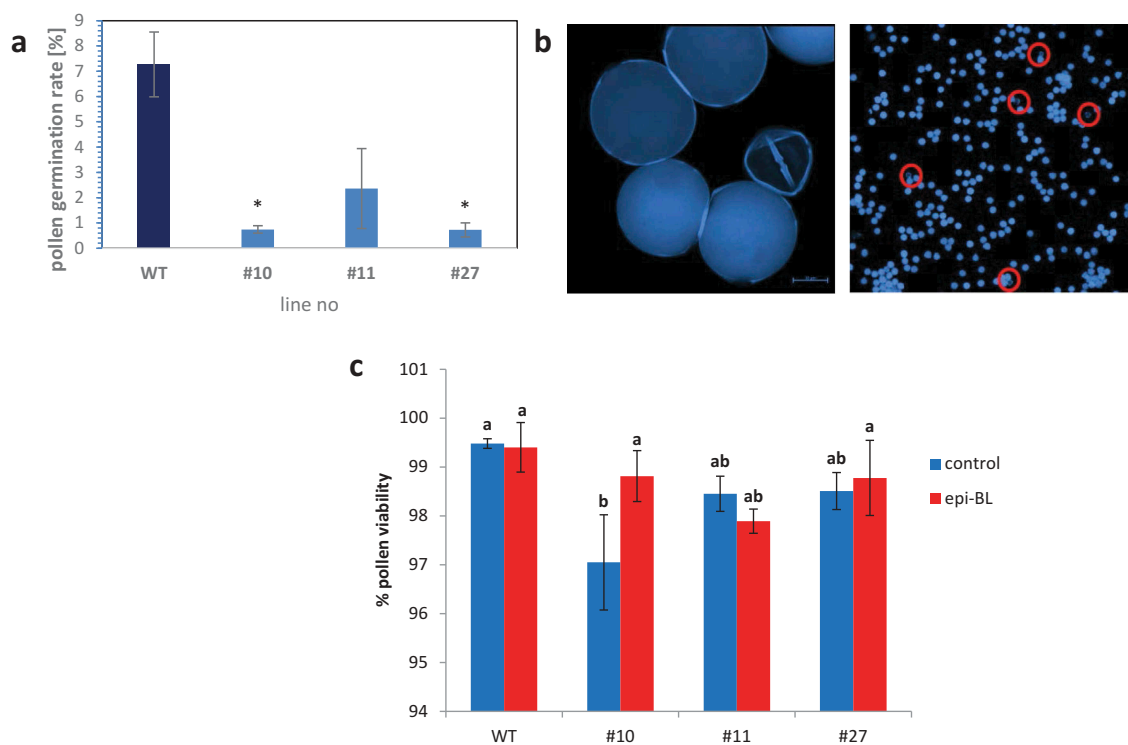
We generated *SISUT2* RNAi plants with more severe down-regulation of *SISUT2* expression than the *antisense* plants published in 2006.<sup>6</sup> The phenotypical changes observed in *SISUT2* antisense plants could be reproduced and the degree of inhibition was even stronger and phenotypical changes even more severe. Whereas *SISUT2* antisense plants showed normal growth,<sup>6</sup> the RNAi plants were dwarfed with significantly reduced plant height (Figure 1).

The level of soluble sugars was significantly reduced only in one strongly inhibited *SISUT2* antisense line #48.<sup>6</sup> In case of *SISUT2* RNAi tomato plants, the reduction of soluble sugars, mainly hexoses is more prominent: in two out of



**Figure 1.** Phenotype of *SISUT2*-RNAi plants.

(a) *SISUT2*-RNAi tomato plants are smaller than WT plants. (b) Plant size of *StSUT2*-RNAi plants is reduced compared to tomato WT plants 8 weeks after transfer to soil. \*\*\* $p < .001$ ; \*\* $p < .01$ . (c) The content of soluble sugars in source leaves of *SISUT2*-RNAi plants is reduced compared to WT plants. \*\*\* $p < .001$ .



**Figure 2.** Pollen germination rate and vitality.

(a) The pollen germination rate of *SISUT2*-RNAi plants is significantly reduced. Asterisks indicate significant differences between WT and RNAi lines (\* $p < 0.05$ ). (b) Viability of pollen was analyzed by aniline blue staining. Viable pollen grains show bright blue fluorescence whereas non-viable pollen are not stained (red circles). (c) Pollen viability of all tested *SISUT2*-RNAi plants is reduced compared to WT pollen. The viability can be rescued by epi-brassinolide treatment in one out of three transgenic lines. A Tukey HSD test was performed with  $\alpha = 0.05$ .

four transgenic lines was only 50% of the WT sugar content, which was mainly due to a reduction in glucose and fructose (Figure 1c). It is therefore unlikely that *SISUT2* promotes phloem loading as shown for *SISUT1*,<sup>6</sup> whose inhibition is leading to high accumulation of soluble sugars in leaves ( )

Consistently to earlier observations,<sup>6</sup> the RNAi plants revealed reduced pollen germination rate and reduced pollen viability (Figure 2a). In order to test whether reduced germination rate is simply due to reduced pollen vitality, aniline blue staining of pollen was performed (Figure 2b). Only viable pollen show bright blue fluorescence after staining. Slightly reduced viability of pollen was confirmed for all tested RNAi lines (Figure 2c). Flowers, that have been sprayed with 1  $\mu$ M epi-brassinolide for a period of 2 weeks show a partial rescue of this defect; differences between the pollen vitality of WT and transgenic plants are slightly diminished by the epi-BL treatment, but the *SISUT2* RNAi plants do not reach WT levels (Figure 2d).

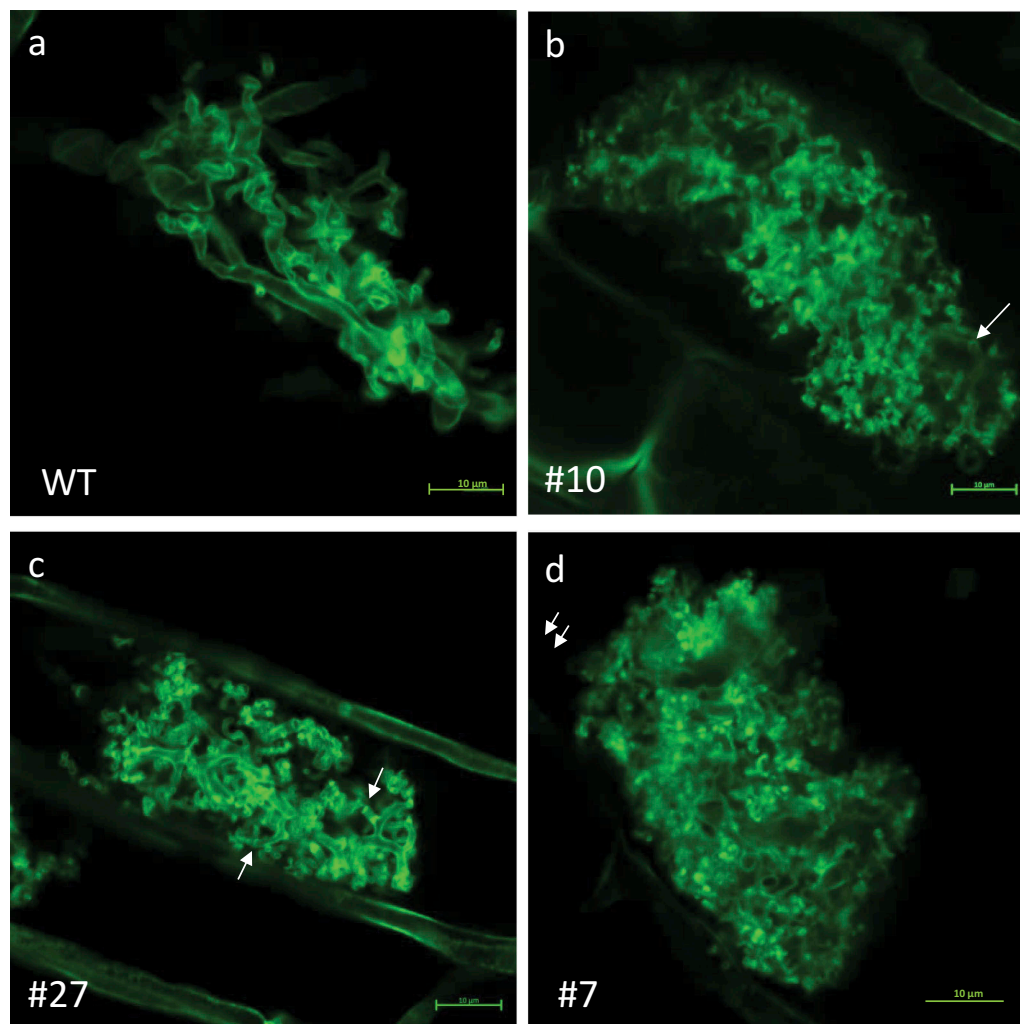
In *SISUT2* silenced plants, AM fungi show increased mycorrhization, while the growth promoting effect of AM fungi on tomato WT plants vanishes.<sup>3</sup> The newly generated *SISUT2* RNAi tomato plants were inoculated with *Rhizoglyphus irregularis*

(Figure 3). At first view, the arbuscules in *SISUT2* RNAi roots (Figure 3b–d) look bushier than in corresponding WT roots (Figure 3a). Increased RNA accumulation of the fungal *RiGAPDH* and the arbuscule-specific phosphate transporter gene *SIPT4* of tomato (Figure 4a) confirmed the increased mycorrhization formerly seen in the roots of the *SISUT2* silenced plants.<sup>3</sup> Quantification of the diameter of the finest tubular branches of arbuscules revealed significantly reduced tubule diameters in *SISUT2*-RNAi plants line #11 and #27 (Figure 4c). At the same time, a high number of septate hyphae were observed in *SISUT2*-RNAi line #27 (Figure 4b).

In contrast to *SISUT2* antisense plants, the fruit yield of *SISUT2*-RNAi plants was not reduced and fruits are not sterile. The number of fruits per plant is slightly increased whereas the total fruit yield was slightly lower (data not shown) indicating smaller fruits.

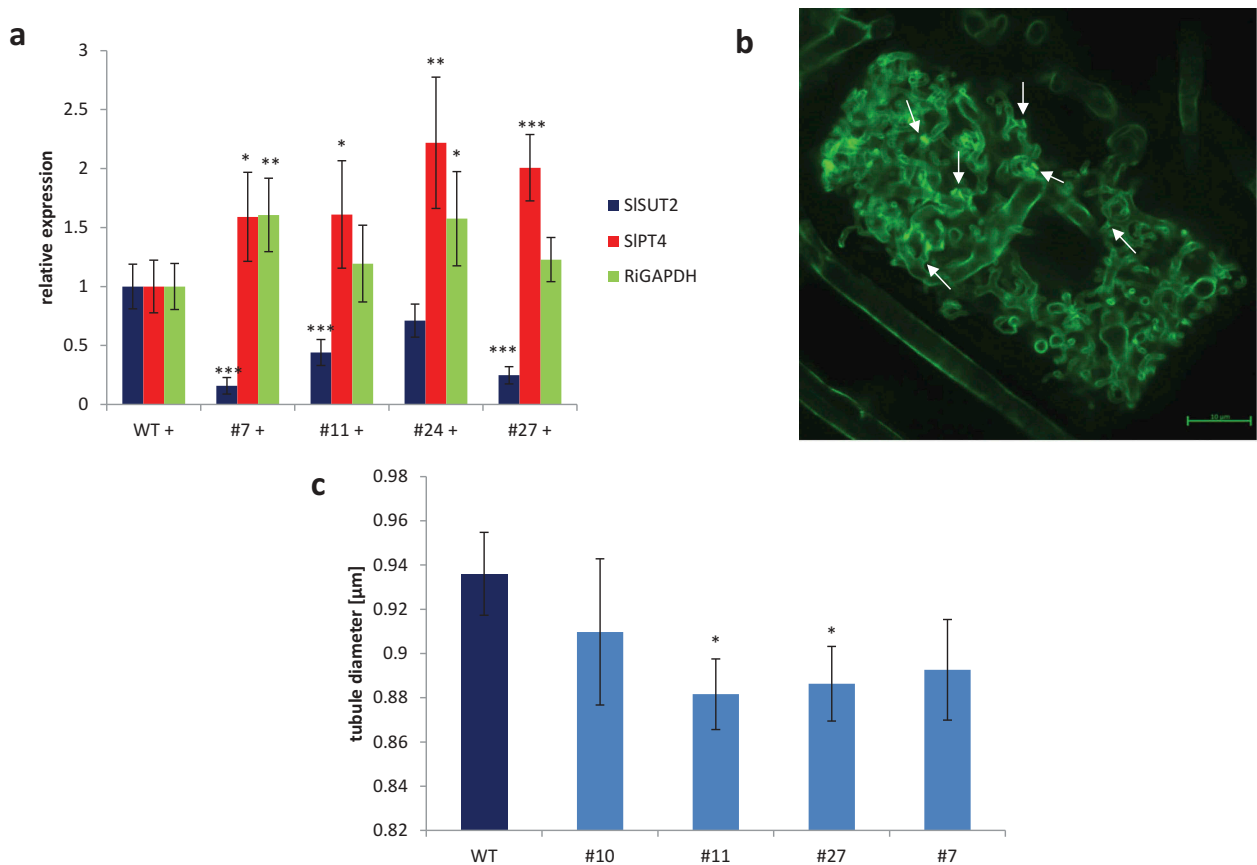
## Discussion

During the last years, evidences accumulate that brassinosteroid (BR) signaling is linked to carbon partitioning and sugar



**Figure 3.** Arbuscular morphology.

Colonized roots of tomato WT (a) and *SISUT2*-RNAi plants (b–d) were stained with WGA-Alexa488. Arbuscules of *SISUT2*-RNAi plants appear bushier and the diameter of finest arbuscular branches is significantly reduced in line #11 and #27 (see results of quantification in Figure 4c).



**Figure 4.** Mycorrhization.

(a) Real time PCR confirmed increased mycorrhizal colonization and function. Reduced *SISUT2* expression (blue) is accompanied by increased gene expression of the fungal *RiGAPDH* (green) or the mycorrhization-specific phosphate transporter gene *SIPT4* (red). \*\*\* $p < .001$ ; \*\* $p < .01$ ; \* $p < .05$ . (b) Arbuscules of *SISUT2*-RNAi #27 show a high number of septa (arrows). (c) Tubule diameter of arbuscule branches is reduced in *SISUT2*-RNAi plant. The diameter of the finest arbuscular branches was analyzed using the confocal Zeiss software ZEN 2.0 blue edition. In case of WT plants, 15 tubules from 4 different arbuscules in 8 different plants were measured ( $n = 480$ ), in case of transgenic *SISUT2*-RNAi plants, 15 tubules from 4 different arbuscules in 3 different plants per transgenic line were measured ( $n = 180$ ). The tubule diameter of finest arbuscular branches was significantly reduced in two out of four *SISUT2*-RNAi plants (\* $p < .05$ ).

availability. Tomato mutant plants defective in BR biosynthesis (*dx*) show reduced levels of sugars and starch in leaves and reduced dry matter content in fruits.<sup>7</sup> BR application to leaves partially rescued this phenotype.<sup>7</sup> In sugar cane, down-regulation of the LRR-receptor kinase and brassinosteroid co-receptor BAK1 affects carbon accumulation. ScBAK1 is mainly expressed in the bundle sheath cells of leaves and its expression is correlated with high levels of sugar.<sup>8</sup> The photomorphogenic mutant *bsl1* (*brassinosteroid, light and sugar1*) from *Arabidopsis* is disturbed not only in its BR response but also in sugar sensitivity, causing a dwarfed phenotype in the light and delayed bolting. The *bsl1* mutant is hypersensitive to metabolizable sugars. The short root and short hypocotyl phenotype together with the sugar hypersensitivity could be rescued by BR application.<sup>9</sup> It is assumed that *bsl1* integrates light, hormone and sugar signaling. The BR regulated gene *EXORDIUM-LIKE 1* is also induced by sugar starvation, prolonged darkness and anoxia. The carbon status in leaves also affects EXL1 protein levels suggesting a role in the carbon starvation response in order to maintain biomass production and survival even under carbon or energy limited conditions.<sup>10,11</sup>

Based on these results, a hierarchical model of sugar and brassinosteroid signaling was postulated also regarding

hypocotyl graviresponse of etiolated *Arabidopsis* seedlings.<sup>1</sup> BR deficiency induced by brassinazole treatment also revealed a link between BR-promoted plant growth and the availability of carbohydrates and energy in *Arabidopsis*.<sup>10</sup> A transcriptomic study suggests that BRs can stimulate both anabolic and catabolic pathways.<sup>10</sup> In some of the BR defective mutants exogenous sugar application antagonizes BR inhibition.<sup>7,9,11–17</sup>

Analysis of BR deficient mutant plants that are devoid of constitutive photomorphogenesis and display a dwarfed phenotype like *cpd* or show reduced expression of the sterol reductase DIMINUTO1 (*DIM1*) revealed altered carbohydrate accumulation and reduced starch levels.<sup>18</sup> Interestingly, these BR defective mutant plants also show impaired carbohydrate uptake and altered enzyme activities and expression patterns of cell wall invertases, sucrose synthases and beta-amylases. This suggests that BR biosynthesis and function are required for carbon uptake and carbohydrate metabolism. The reduced growth of BR defective mutants might be explained by reduced carbon availability and/or photosynthetic capacity.<sup>18</sup>

*SISUT2* directly interacts with components of the BR signaling pathway, namely, the BR co-receptor BAK1 and the BR signaling inhibitor MSBP1.<sup>3</sup> Whereas *SISUT2* expression is increased in response to biotic interactions, the expression of MSBP1 is reduced according to the TomExpress



database.<sup>5</sup> Interestingly, the overexpression of MSBP1 and roots treated with the BR biosynthesis inhibitor Brassinazole reveal the same effect on arbuscular morphology; both reduced the diameter of arbuscular branches and arbuscules appeared bushier. In parallel, the exchange capacity of nutrients between host plants and mycorrhiza seems to decline;<sup>5</sup> mycorrhizal plants do not show an increase in biomass, nor an increase in mineral nutrients such as Mn, K, Ca, Fe, Zn and P in response to mycorrhization.<sup>5</sup> Unexpectedly higher branching of arbuscules is paralleled by diminished nutrient exchange and membrane transport between plants and fungi.

*SISUT2* RNAi plants confirmed previous observations with *SISUT2* antisense plants and it became obvious that *SISUT2* silencing is accompanied with a dwarfed phenotype and reduced pollen germination and viability leading to male sterility of flowers. This characteristic phenotype could partially be rescued by BR treatment (Figure 2d).

Arbuscular architecture responded similarly to silencing of *SISUT2* and *MSBP1* overexpression or Brassinazole treatment<sup>5</sup> (Figures 3 and 4). Here again, the decrease of the diameter of arbuscular branches is accompanied by the loss of the plant's growth benefit by root colonization.<sup>3</sup> This suggests contrary functions of *SISUT2* and *MSBP1* on mycorrhizal colonization and development within the plant root. Thus, the *SISUT2*-silencing effects on mycorrhization efficiency cannot exclusively be explained by its putative retrieval function at the periarbuscular membrane. It cannot be excluded that *SISUT2*-mediated effects on mycorrhization are also due to de-regulation of BR signaling. Further experiments are needed to analyze whether brassinosteroids affect carbon partitioning between the host plant and the fungus.

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